REMARKS

The Amendments

Claim 5 is amended to make it dependent upon claim 1, the scope and meaning of the claim is unchanged. The amendment does not narrow the scope of the claims and was not made for reasons related to patentability.

Request to Withdraw Finality of Office Action

Contrary to the allegation in the Final Office Action, the new grounds of rejection made therein were not necessitated by applicants' amendments. The withdrawal of the previous grounds of rejection was a result of the inapplicability of the originally cited prior art, as pointed out in applicants' arguments, not due to their amendments. Applicants' amendments could not have necessitated withdrawal of the previous rejections because the amendments broadened the claimed subject matter. If the original rejections had been properly applicable to the original claims, they would remain applicable to the instant claims because the instant claims wholly encompass the subject matter of the original claims.

Because applicants' amendments did not necessitate the totally new grounds of rejection herein, the Office Action was not properly made Final and the finality thereof should be withdrawn (unless, of course, the application is allowed rendering the need for withdrawing finality moot). Applicants must be afforded a full and fair opportunity to respond to new grounds of rejection not occasioned by their action before a Final Office Action. Applicants can only be afforded that opportunity here if the finality of the previous Office Action is withdrawn.

The Restriction Requirement

Applicants respectfully renew their traversal of the restriction requirement restricting claims 3 and 5-15. Applicants acknowledge the statements in the Office Action regarding applying the Unity of Invention standard rather than the MPEP standard. But the Unity of Invention standard is less restrictive than the MPEP standard and applicants' traversal is even more convincing under this standard.

Claim 3 and claims 5-15 clearly have Unity of Invention with claims 1, 2 and 4 because they all are based on the encased monolithic sorbent as recited in claim 1. Claim 3 is directed to a method of using this defined sorbent and claims 5-15 are directed to a method of making this defined sorbent (claim 5 is made dependent on claim 1 by the above amendment to make this even more clear).

The application of the standard as set forth in the Office Action is not proper. Under the reasoning set forth therein, any claim merely alleged to be rejectable over prior art could be restricted from any other claim. The definition of "special technical features" relates to aspects of the invention which applicants view as distinguishing the art. The Office Action admits that the sorbent as recited in applicants' claim 1 is a special technical feature. This feature is shared by all the instant claims and, thus, there is unity of invention. That they are rejected as purportedly anticipated or obvious (see traversal thereof below) does not change this fact.

Applicants' previous arguments based on the MPEP standard even further support withdrawal of the restriction. If it is not supported by the tougher MPEP standard, it surely is not supported under the Unity of Invention standard.

For the above reasons, it is urged that the restriction should be withdrawn and all the claims examined.

The Rejection under 35 U.S.C. §102

The rejection of claims 1 and 2 under 35 U.S.C. §102, as being anticipated by WO 94/19687, is respectfully traversed.

WO 94/19687 discloses porous ceramic shaped bodies for use as a substance separating medium, particularly in chromatography columns or cartridges. The reference also discloses that the porous ceramic shaped bodies can be surface-modified. At page 7 and in Figure 1, the reference depicts a porous ceramic shaped body which is covered by liquid-impermeable Teflon sleeve; element 2 in the drawing. This is covered by a pressure-resistant sleeve (i.e., "Druckmantel" (element 3 in the drawing)), which is particularly of a metal, to provide a rigid, pressure-resistant structure. See the discussion of WO '687 on page 1 of the instant specification. It is alleged in the Office Action, based on U.S. Patent No. 4,556,538, that the "impermeable Teflon material of WO 94/19687 reads on Applicants' liquid-impermeable, pressure-resistant plastic casing because it is known in the art of chromatography that Teflon is pressure resistant."

Applicants respectfully disagree that the '538 patent shows that the Teflon sleeve as used in WO 94/19687 is known to be pressure-resistant. In fact, the art considered as a whole shows the opposite. The cited portion of the '538 patent discloses that the pressure-resistant column is of stainless steel, Teflon, acrylic resin, polyethylene or glass. If the column is of Teflon in the '538 patent, it would have to be of a different structure than in WO '687. For example, it would have to be a much thicker layer of Teflon. Or it could be provided with a rigid, e.g., metal, covering as in WO '687, in which case it is the covering, not the Teflon, which provides the pressure-resistance. One of ordinary skill in the art would know that such a pressure resistant column could not be provided merely with the thin Teflon sleeve such as shown in WO 94/19687 since a thin layer of Teflon does not provide a rigid structure. This is clearly shown in WO '687

by the fact that an outer pressure-resistant covering, i.e., the Druckmantel (3), must be provided around the thin Teflon coating to provide a pressure-resistant structure. WO '687 specifically states that it is this thicker outer covering, not the Teflon sleeve, which provides the pressure-resistance. The German word "Druck" refers to pressure.

WO '687 also discloses an embodiment wherein a liquid is filled in a gap (i.e., the "spalt" element 8) between the Teflon coating and the pressure-resistant covering to ensure a close fit of the Teflon sleeve to the sorbent. This would not be possible unless the Teflon sleeve was flexible and, thus, not pressure resistant.

Accordingly, it is believed to be evident from the art that the Teflon sleeve in WO '687 is not a "pressure-resistant plastic casing" as recited in the instant claims. Thus, WO '687 does not meet all elements of the claims and cannot anticipate the claims under 35 U.S.C. §102. The rejection should, therefore, be withdrawn.

The Rejection under 35 U.S.C. §103

The rejection of claim 4 under 35 U.S.C. §103, as being obvious over WO 94/19687 in view of Nakanishi (U.S. Patent No. 5,624,875), is respectfully traversed.

The discussion of WO '687 above is incorporated herein by reference. Nakanishi was cited for its teachings regarding pore types and size of a sorbent material. As applicants have previously established, Nakanishi teaches nothing about encasing such a sorbent material and particularly not encasing in a "liquid-impermeable manner by a pressure-resistant plastic casing." Thus, Nakanishi provides no motivation to modify the encasing structure of WO '687. WO '687, likewise, provides no motivation to provide a sorbent encased in a "liquid-impermeable manner by a pressure-resistant plastic casing." In the absence of any such motivation, the claimed

invention could not have been obvious to one of ordinary skill in the art from these references. Thus, the rejection under 35 U.S.C. §103 should be withdrawn.

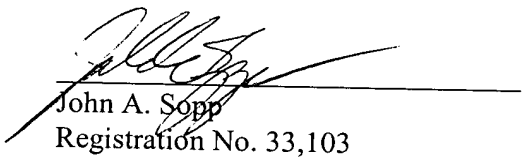
Miscellaneous

Copies of the two articles discussed in the specification and requested in the Office Action are provided herewith.

It is submitted that the application is in condition for allowance. But the Examiner is kindly invited to contact the undersigned to discuss any unresolved matters.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,



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Date: November 14, 2002
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Amend claim 5 to read as follows (a marked up version of the amended claims is in an appendix attached hereto):

5. (Amended) A method of ~~encasing a~~ making an encased monolithic ceramic sorbent according to claim 1, comprising:

a) providing a monolithic ceramic sorbent comprising at least one porous ceramic moulding, and

b) providing a ~~tightly fitting~~ liquid-impermeable, pressure-resistant fitted polymer casing around the ceramic sorbent.

CORRESPONDENCE

Continuous Rods of Macroporous Polymer as High-Performance Liquid Chromatography Separation Media

Sir: While synthetic polymers can be fashioned into almost any shape, small diameter spherical beads are essentially the only form of polymers used in modern column liquid chromatography. The only significant exception may be the cartridges containing sheets of planar chromatographic separation media introduced recently by Millipore, Säulentechnik Knauer, BioRad, Cuno, and others.[1]

A theoretical analysis of conditions influencing the efficiency of a column in the separation of macromolecules with molecular weight exceeding 10^5 revealed that the optimum size of porous particles in the packing medium is about 1 μm.[2] When this size is reached, the slow diffusion of solutes within the pores does not restrict the separation quality. While even smaller sizes would theoretically be desirable, current limitations in the technology, including packing problems, column back pressure, dead volumes in detection instruments and connecting tubes, etc., make the concept of using very small beads unpractical. Current trends seem to favor particle sizes in the range 3–10 μm.

In order to improve the kinetics of the separation process, perfusion chromatography, which is based on the use of packings with very large pores of up to 1 μm, was developed.[3,4] These large pores allow at least part of the mobile phase to flow through the beads, rather than around them, therefore improving the kinetics of separation in the interior of the beads by reducing the diffusional path length. It has been estimated that approximately 5% of the mobile phase flows through the porous particles in a perfusion column.[3]

The perfusion concept has been further improved in some ways by the development of high-performance membrane chromatography in which *all* of the mobile phase flows *through* the flat macroporous polymer body, which may be 2-mm thick and is held in a cartridge or cell.[1,5] The separation proceeds by gradient elution and the method is useful in the chromatographic modes based on the on–off principle.

Hjerten has recently described a column consisting of a continuous strongly compressed plug 30-mm long and 6-mm diameter based on solvent-swollen poly(acrylic acid-*co*-methylenebisacrylamide). Despite the lack of a permanent pore structure, this material could be used in the separation of proteins by ion-exchange mechanism[6] provided the flow rate was kept near 0.5 mL/min, resulting in a column back pressure of 1.4 MPa. This finding is interesting though more versatility would likely have been achieved had a permanent macroporous structure been incorporated in the polymer plug.

The main advantage of both the macroporous membrane and the compressed plug approaches is that the separation occurs as *all* the mobile phase flows *through* the porous structure of the separation medium. This reduces the elution pathway and facilitates diffusion in and out of small porous areas. However, the macroporous membranes have limited capacities and can only be used in a specialized form of separation. Similarly, the compressed plugs may not be suitable for use in solvents of widely different polarities as they do not possess a macroporous structure and they depend on swelling phenomena for their operation.

This report deals with the preparation of a novel continuous bed column that incorporates both macroporosity and capacity. These rod-shaped columns, consisting of a single "molded" piece of macroporous polymer, can be used in most chromatographic modes and offer a tempting alternative to the standard columns packed with particles.

EXPERIMENTAL SECTION

Preparation of a Continuous Rod of Porous Polymer. The continuous porous polymer rod was prepared by an in situ polymerization within the confines of the tube of a chromatographic column 30 mm × 8-mm i.d. The 40:60 vol % mixture of monomers (glycidyl methacrylate and ethylene dimethacrylate, 60:40 vol %) and porogenic solvents (cyclohexanol and dodecanol, 80:20 vol %) in which azobisisobutyronitrile (1 wt % with respect to monomers) was dissolved, was purged with nitrogen for 15 min and injected into the stainless steel column tube stoppered on one end with a steel nut plug, and the original opening was then closed with a silicon rubber septum. The polymerization was allowed to proceed for 6 h at 70 °C within the column acting as a mold. The stopper and the septum were removed, and the excess polymer at the ends of the "molded" continuous block of porous polymer was detached; the column hardware was then assembled and connected to a HPLC pump. Several 30-mm-long columns were prepared using the same polymerization conditions. The porogenic solvents and other soluble moieties within the porous polymer block were washed out by pumping methanol at a flow rate 1 mL/min for 2 h.

Preparation of Diol-Functionalized Porous Polymer Rod. The epoxide groups of porous polymer rod were hydrolyzed by filling the column with 0.5 mol/L aqueous sulfuric acid then placing it in a water bath at 60 °C for 3 h. The hydrolyzed column was then attached to the chromatograph and washed at a flow rate of 1 mL/min with 100 mL of water, 100 mL of 50:50 water–THF mixture, and 100 mL of THF.

Preparation of Amino-Functionalized Porous Polymer Rod. The epoxide groups of a similarly prepared porous polymer rod were aminated by injection of 2 mL of diethylamine and 3 h heating to 70 °C to afford the corresponding polymer with 1-(*N*,*N*-diethylamino)-2-hydroxypropyl groups.[7] For the chromatographic experiments, the modified column was attached again to the HPLC equipment and washed successively with 100 mL of methanol, 200 mL of 50:50 methanol–water, 200 mL of water, and 200 mL of 0.01 mol/L Tris-HCl buffer solution pH 7.6. Elemental analysis of the resulting polymer indicated that it contained 1.6 mmol of amino groups per gram. There is no difference in the nitrogen content of samples taken from the outer skin and core at the top, center, and bottom of the modified rod. As the polymer containing the (diethylamino)hydroxypropyl groups can complex metal ions,[8] a disk cut in the rod after its removal from the column housing was stained with copper(II). Observation of the disk shows that it is homogeneously blue colored. This is confirmed by examination with a scanning electron microscope which does not show any inhomogeneity across the rod.

Chromatographic Testing of the Porous Polymer Rods. Chromatographic testing was carried out using a Nicolet LC 9560 ternary gradient liquid chromatograph provided with a Hewlett-Packard 1050 UV detector. The samples were injected through a Rheodyne valve loop injector. Following a 10-min elution period with 0.01 mol/L Tris-HCl buffer solution at pH 7.6, a 10 min linear gradient from the starting buffer to 1 mol/L NaCl in the same buffer was utilized for the chromatographic

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separations. The elution profile reflects the gradient used as a change in base line.

RESULTS AND DISCUSSION

The lack of development of continuous bed chromatographic media based on porous polymer rods can be attributed in part to the lack of appropriate technology in the preparation of such materials, and to the fear that extremely high back pressures would be encountered in practical applications. This assumption was based on extrapolation of data obtained from experience with smaller and smaller particulate packings. Early work by Hjerten[6] with compressed plugs of a solvent-swollen hydrophilic polymer has already shown that acceptable back pressures can be achieved at a moderate flow rate. However, this observation made for a swollen material cannot be assumed to hold for a macroporous structure which would normally be expected to be even more suitable for chromatographic applications. The advantages inherent to macroporosity derive from the coexistence of globules and void spaces or pores within the macroporous structure.[9] The globules have a compact core consisting of highly cross-linked material surrounded by a layer of less cross-linked polymer chains. The cores confer rigidity to the material, while the surrounding chains may swell when they are modified or placed in an appropriate solvent. As a result of this local swelling the voids between globules are filled to different degrees depending on the exact conditions. Overall, the swelling has essentially no effect on the actual size of the separation medium but it results in a decrease in pore size.

The pores of a macroporous polymer are interconnected, and therefore the entire structure is permeable to solutes as well as eluting solvents. In addition, control over pore size and pore size distribution, based on the selection of porogenic agents, their content in the polymerization mixture, as well as on the concentration of the cross-linking agent in the monomer mixture, is available for fine tuning of the separation medium.[10]

The approach we use to prepare porous polymer rods is advantageous as both chemical reactivity and macrostructure are built into the material in one simple operation. In addition as the porous polymer rods are prepared in situ within the column tube, by a process somewhat akin to molding which affords a final product having the desired geometry and requiring no tedious packing operation. This is a significant advantage as the efficiency of packed bead columns is known to be affected by packing conditions, including the skills of the operator, as well as numerous other variables.[11] Due to their high degree of cross-linking, the macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) rods we prepare are essentially incompressible hard materials that fully occupy the tube space. Their inner macroporous structure is readily controlled by adjustments to the composition of the polymerization mixture.[10] Since the back pressure may be expected to be inversely related to pore size, we have chosen, for the first illustration of this novel approach, a composition of the polymerization mixture which provides a final material with rather large pores and a specific surface area of ca. 10 m^2/g.

The permeability of the porous polymer rod is confirmed in chromatographic measurements with the hydrolyzed diol-functionalized column in THF. The retention time for both benzene and polystyrene (MW 2.9×10^6) at a flow rate of 2 mL/min are very similar, 0.63 and 0.60 min, respectively, and no exclusion effects are seen. The resulting chromatographic peaks are quite narrow. These findings suggest that the rods prepared under our experimental conditions contain almost no micropores.

The back pressure vs flow rate dependency was of prime interest. Figure 1 shows that the relationship is almost linear

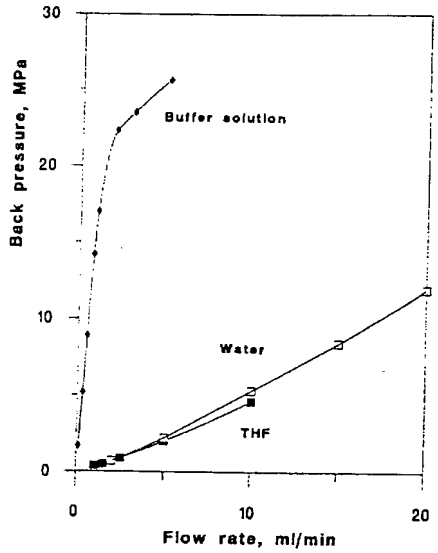

Figure 1. Effect of flow rate on back pressure in the diol (THF and water) and aminofunctionalized (buffer solution) porous polymer rod column. Conditions: column 30 mm × 8-mm i.d. Mobile phase: water, THF, and 0.01 mol/L Tris-HCl buffer pH 7.6.

for flow rates up to 20 mL/min and proves the absence of compression of the rod. The back pressure in the hydrolyzed column is somewhat higher for water than is the case for THF due to effect of solvent viscosity. The switch from water to THF and back neither changes the structure of the rod nor causes any mechanical damage. Repeated permeability measurements in both water and THF provided identical results. These correlate to chromatographic results achieved with beads of similar composition packed in a column.[12]

For the amine-modified columns, used with the buffer solution as mobile phase, the curve is steeper but linear up to almost 2 mL/min. The higher slope of the back pressure vs flow rate for this ion-exchange column illustrates the effect of swelling. The rod is located in the tube and cannot increase its diameter; therefore, swelling results in a decrease in the overall permeability of the medium. Surprisingly, at higher flow rates the column does not plug as is observed for classical columns packed with polymer beads,[13] but the slope of the back pressure vs flow rate curve decreases dramatically.

For the diol column, the retention volume of benzene in THF does not depend on the flow rate and is 1.26 mL on average. The volume of the empty column is 2.51 mL and the extra column volumes for connections and tubings amount to 0.11 mL. From these data, the porosity of the rod is calculated to be approximately 50%, a value which is in good agreement with that expected from the amount of porogens used. This porosity suggests that, on average, only half of the cross section of the continuous chromatographic rod is filled with the solid polymer globules. The other half constitutes the free pores through which the mobile phase can flow.

Although the calculation is not relevant to ion-exchange chromatography but to size exclusion chromatography which is our future target, a rough estimate of the plate number for the 30-mm-long continuous rod column, using the sharp benzene peak in THF at a flow rate 1 mL/min, is 3500 plates which corresponds to 117 000 plates/m or a HETP of about 9 μm. This value compares very favorably to any bead-packed polymer column currently available.

A few model separations of protein mixtures using typical ion-exchange chromatography techniques illustrate the good separation properties of the amino functionalized porous rod

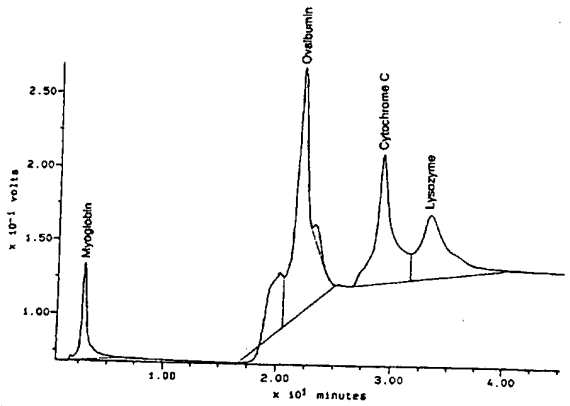

Figure 2. Ion-exchange chromatograph of model protein mixture in the porous polymer rod column. Conditions: column 30 mm × 8-mm i.d., poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) modified with (diethylamino)hydroxypropyl groups. Mobile phase: 10 min 0.01 mol/L Tris-HCl buffer solution at pH 7.6, followed by 10 min gradient of the same buffer from 0 to 1 mol/L NaCl, flow rate 0.5 mL/min, UV detector 218 nm, injection 2 μL of a solution containing total 16 mg/mL proteins.

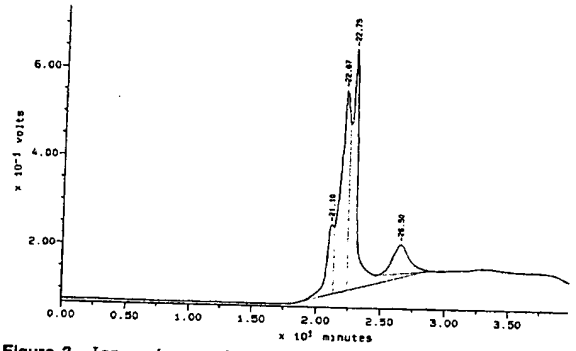

Figure 3. Ion-exchange chromatograph of egg white protein in the porous polymer rod column. Conditions: injection 0.1 μL of a solution containing 16 mg/mL egg white; other conditions see Figure 2.

polymer columns (Figures 2–4). The myoglobin, use as a nonretained compound, elutes with the starting buffer prior to the start of the gradient. Its retention volume is 1.43 mL, in good agreement with the retention volume of benzene in the hydrolyzed diol column. Preliminary measurements of albumin recovery obtained by a comparison of the weight of injected protein and the amount determined by measurements of the UV absorption in the recovered solution shows better than 95% recovery in all cases. This value did not change in subsequent measurements.

In addition to the considerable advantages resulting from their in situ preparation within the confines of a chromatographic column, the polymerized porous polymer rod columns benefit from increased reproducibility as confirmed in parallel studies with different columns prepared under identical conditions. The chromatograms acquired on columns prepared from the same mixture are all equivalent. As the chromatographic properties of the rod column did not change over a period of 5 weeks of daily use, durability of the column can be assumed to be sufficient.

In contrast to bead materials which often require considerable size classification before use, the rod approach affords no wastes. Almost any monomer can be used, including those that are water soluble and would therefore not be useful in

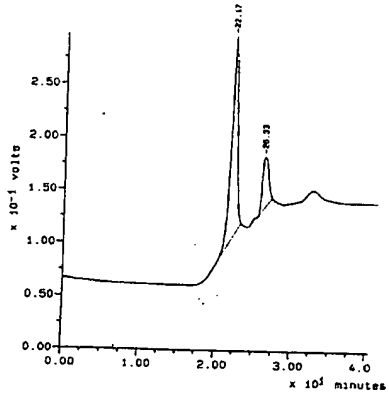

Figure 4. Ion-exchange chromatograph of chicken egg albumin in the porous polymer rod column. Conditions: injection 2 μL of a solution containing 8 mg/mL ovalbumin; other conditions see Figure 2.

suspension polymerizations using water as the continuous phase. The copolymerization approach allows access to a variety of primary functionalities without additional chemical modification steps.

While this report is only preliminary, it demonstrates the viability of polymerized porous rod separation media. We are currently refining the preparation methods, studying structure–chromatographic properties relationships, and working on continuous columns for other chromatographic modes and prepared from different comonomers.

ACKNOWLEDGMENT

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application to chromatogram pairs, the adequacy of the retention time observations (sometimes overlooked by data users) are included. Within these limits, each value can be a useful index of complications eliminating the need for the sometimes severe censoring of data.

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The author wishes to thank L. H. Wright and J. V. Daughtridge for their generosity and excellent work in obtaining the chromatograms. This article has not been subjected to Agency review and, therefore, does not necessarily reflect the views of the Agency. No official endorsement should be inferred.

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Note

High-performance liquid chromatography on continuous polymer beds

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Current research in this laboratory is directed toward the investigation of various possible means to increase performance in electrophoresis and chromatography. These methods are studied in parallel because the separation mechanisms are analogous[1–3], which means that solutions of methodological problems in electrophoresis are often applicable to analogous problems in chromatography and *vice versa*. In the chromatographic field we give an high priority to practical solutions (with the aid of theoretical considerations) of the following fundamental questions:

(1) Is it possible to design chromatographic beds such that the resolution is independent of or even increases with an increase in the flow-rate and bead size? Classical chromatographic theory says no. However, the experiments described in refs. 4–6 and forthcoming papers show that compressed beds of non-porous agarose beads have the desired unique relationship between the resolution, flow-rate and bead-size.

(2) Is it possible to design a chromatographic bed by bulk polymerization directly in the chromatographic tube? It has been taken for granted that a chromatographic bed must be built up of granulated particles, preferably in spherical form. Even when the spheres are monodisperse the packing is never perfect. The theoretical maximum resolution can therefore never be attained. Further disadvantages of packed beds are the time-consuming and expensive steps required for preparation of the beads, the sieving of the beads to select the desired size (if not monodisperse in the preparation) and the packing of the column with the beads.

A continuous gel plug with channels sufficiently large to permit an hydrodynamic flow might be the ideal chromatographic column. One could then expect the zones to be almost as sharp as those obtained in agarose or polyacrylamide gel electrophoresis. Unfortunately, the latter continuous gels cannot be used for chromatography, since they collapse when pressure is applied, *i.e.*, water cannot be pressed through them. However, more than 20 years ago we prepared a polyacrylamide gel (cross-linked in a special way) directly in a glass tube and on this gel plug separated monomers and dimers of albumin by molecular-sieve chromatography. The flow-rate was relatively low, which limited its usefulness. We have now resumed these experiments and at the same time have tried to improve the mechanical properties of the gel plug so that it will withstand higher pressures and thereby permit higher flow-rates. The polymerization technique is still under development and will be published else-

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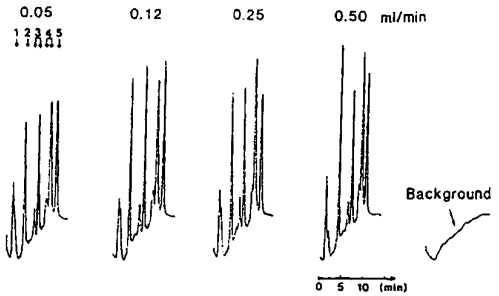

Fig. 1. High-performance cation-exchange chromatography of model proteins on a compressed continuous gel at the flow-rates indicated.

where. However, since the experiments are promising we present some preliminary results in order to focus interest on columns of continuous polymers.

EXPERIMENTAL AND RESULTS

The amphiphilic, macroporous gel plug consisted of a copolymer of acrylic acid and N,N'-methylenebisacrylamide. The gel plug was strongly compressed to a bed height of 3 cm (the importance of compressing a bed to increase its resolution is discussed in ref. 7). The diameter of the gel plug was 0.6 cm. This gel column was utilized for a cation-exchange chromatography experiment performed in the following way.

After equilibration with 0.01 *M* sodium phosphate, pH 6.4, a 40-μl sample [about 10–15 μg of each of the proteins alcohol dehydrogenase (1), horse skeletal muscle myoblobin (2), whale myoglobin (3), ribonuclease A (4) and cytochrome *c* (5)] was applied. Elution was performed with a linear gradient formed from the equilibration buffer and 0.01 *M* sodium phosphate, pH 6.4, containing 0.25 *M* sodium chloride. The flow-rates were 0.50, 0.25, 0.12 and 0.05 ml/min. The gradient volume was constant at 5.0 ml. The chromatograms are shown in Fig. 1. The relationship between the pressure and flow-rate is presented in Fig. 2.

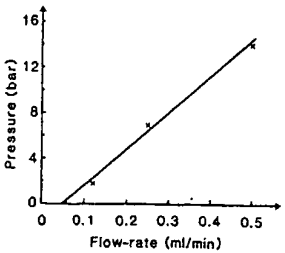

Fig. 2. The relationship between the flow-rate and pressure for the gel column used in the experiment shown in Fig. 1.

DISCUSSION

From Figs. 1 and 2 one can draw several conclusions:

(1) It is possible to prepare by bulk polymerization a continuous gel with channels large enough to permit passage of buffer when a pressure is applied to the bed.

(2) The bed is sufficiently rigid to give high flow-rates at moderate pressures.

(3) It is possible to prepare directly in the chromatographic tube a gel bed useful for ion-exchange chromatography. No subsequent step is required for attachment of ligands.

(4) The resolution on the continuous gel is roughly independent of the flow-rate, which is in sharp contrast to what is observed on columns of macroporous beads[8]. The reasons are probably that a gel plug has a more homogeneous structure than a packed bed of beads and that the gel plug was compressed, which, analogously to a compressed bed of agarose beads[7], has a favourable effect on the resolution. It is also likely that the gel plug is non-porous, *i.e.*, the "walls" of the channels in the gel are impermeable to proteins, which in combination with compression of the bed gives a resolution with the attractive flow-rate dependence mentioned above[4–6].

We are working on the preparation of continuous polymer beds for both hydrophobic-interaction and anion-exchange chromatography as well as for other kinds of chromatography.

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